DETERMINATION OF THE CRITICAL TIME WHEN LEVELS OF THE CYANIDE POTENTIAL IN CASSAVA ARE AT PEAK CONCENTRATION

Weru, S.M., Filippo de Monte, Kanamu, J.M., Farra, C.N., Mwiti, G.M. and Rewe, M.N.
Kenya Agricultural and Livestock Research Organization Mtwapa, P. O. Box 16-80109 Mtwapa.
CAST NGO, P. O. Box 251-80108, Kilifi
Ministry of Agriculture, Fisheries and Livestock, P. O. Box 19-80108 Kilifi
Kenya National Farmers Federation, P. O. Box 19-80108 Kilifi
Email: sweru81@yahoo.com

ABSTRACT
Cassava (*Manihot esculanta* Crantz) is the second most important food crop and a main source of income for the rural communities with potential for industrial use in the coastal region of Kenya. Cassava, now widely grown in the coastal region of Kenya is a domesticated plant derived from one or more species of the Genus Manihot in the Euphorbiaceae family. Cassava contains naturally occurring, but potentially toxic compounds called cyanogenic glycosides, which release hydrogen cyanide (HCN) as a result of enzymatic hydrolysis following maceration of the plant tissue. The objective of the study was to sustain and enhance the food security and livelihood of coastal lowland farmers and processors by assisting them to successfully produce/trade/export their produce in compliance with food safety standards for cassava and cassava products. The study was to conduct a scientific assessment to determine ways along the food chain/commodity pathway to minimize the hydro cyanic acid content in cassava and its products and thus provide methods of meeting food quality standards requirement. This was achieved by determining, through study and analysis, the effect on cyanide content based on agronomic factors (e.g. cultivars, stress,), agro ecological zone (CL 3, CL 4, and CL 5) and harvest/post-harvest practices i.e. age at harvest. The study was conducted in 2016 in Kilifi County in three agro ecological zones. They are coastal lowlands 3, 4 and 5. The genotypes studied and analyzed are Tajirika (improved), Shibe (improved), Karembo (improved) and Kibandameno (traditional). The analysis was carried out at 5, 8 and 11 months after planting. At five months after planting in loamy soils for the Kibandameno variety, the cyanide level is recorded 0.5mg/kg while at 11 months after planting in the same soils and AEZ's, the level is 0.33mg/kg. At five months after planting, Tajirika, Shibe and Kibandameno varieties in CL5 recorded cyanide levels of 0.44 and 0.48mg/kg respectively in sandy loam soils. In the eleventh month, Shibe and Kibandameno varieties in the same soil type recorded 0.41 and 0.45 mg/kg, respectively. There is a slight indication that as the plant ages the levels of the cynogenic potential (CNP) declines.
Keywords: Cassava, Domesticated, Genotypes, Species, Cyanogenic glycosides, Enzymatic hydrolysis

INTRODUCTION

Cassava is the second most important food crop and a main source of income for the rural communities with potential for industrial use in the coastal region of Kenya. Cassava, now widely grown in the coastal region of Kenya is a domesticated plant derived from one or more species of the Genus Manihot in the Euphorbiaceae family (Allem, 2002). Cultivated cassava is *Manihot esculanta* Crantz. Since its domestication thousands of years ago in the Amazon region, cassava is now spread around the world and is widely cultivated for consumption in the tropics and sub-tropic. Thousands of cultivars are in existence most adapted to local conditions. Cassava is a very flexible crop that grows well under marginal conditions where other crops could not survive and does not require a large amount of agricultural input (e.g., water, fertilizer and pesticides). Most cassava varieties are drought tolerant and are naturally tolerant to acidic soils making cassava a fundamental food security component in marginal agricultural land. Cassava is grown primarily for its starchy tuberous roots, which are important staple for more than 800 million people, mostly in sub-Sahara but also in other parts of Africa, Asia and South America (Burns et.al. 2010). It has also become a very important root crop in the coastal region where not only has it become a dietary staple but also traded as a raw product or in processed form like chips and flour. Breeding work has been carried out at Kenya Agricultural and Livestock Research Organization (KALRO) Mtwapa for the last ten year and cultivars that are resistant to debilitating biotic stresses due to viral infections have been developed. The traditional cultivars found in the coastal region are inherently low yielding (5 to 9 t ha^{-1}) and are highly susceptible to diseases. However, their dry matter content and low cyanide level produces roots that are quite palatable. These are qualities introgressed in the varieties developed. As such, these developed varieties have dry matter (DM) of over 30% and cyanogenic potential (CNP) of 4.0 in the Piccric acid scale of 1 to 9.

Cyanide forms when cassava is processed; the toxin is volatile and is released into air rather than remaining in the food. Correct processing ensures that the cyanogen content in cassava plants is within acceptable range (Sayre et al, 2011).

The United Nations' Food and Agriculture Organization has established maximum recommended cyanide levels for foods. Classifications of cassava safety limits indicate:

- Mild (safe): 50 mg HCN/kg/fresh peeled storage root;
- Moderately poisonous: 50-100 mg HCN/kg fresh peeled storage root;
- Dangerously poisonous: over 100 mg HCN/kg fresh peeled storage root.

Cyanide in Cassava

Cassava belongs to the same sub-family as rubber (*Hevea brasiliensis*) and like rubber contains both cyanogenic glucosides and latex (Jorgensen et al., 2005). Cassava roots contain considerable quantities of cyanide which occurs in the form of cyanogenic glycosides, primarily linamarin and a small amount of lotaustralin (Uyoh et al., 2007). These cyanogenic glycosides break down to release toxic hydrogen cyanide gas during digestion (Poulton, 1988). The consumption of cassava can therefore be harmful to human health. Despite the presence of these naturally occurring toxins, millions of people all over the world have been safely consuming cassava for hundreds of years. The on-going challenge is to ensure that the presence of these cyanogenic glycosides are minimized through proper understanding and control measures through processing to reduce effects of cyanogenic glycoside content of cassava. Roots and leaves contain the highest amount of linamarin (Cereda and Mattos, 1996).

Postharvest Practices

Post-harvest deterioration is the most important cause of loss in cassava production and this is mainly as a result of microbial invasion of the tuber (Okigbo et al., 2009). Post-harvest deterioration can render cassava unpalatable and un-marketable within 24-72 hours (Rielly et al., 2004). Cassava must also be processed before being eaten.

The Amerindians, who first cultivated cassava, over the years, have devised numerous processing techniques not only to increase palatability and extend shelf life, but also to decrease its cyanogenic potential. Today, a great diversity of processing methods are found in the various parts of the world where cassava is consumed (Lancaster et al., 1982). These methods consist of different combinations of peeling, chopping, grating, soaking, drying frying, boiling and fermenting. In Africa where cassava flour is a major product, wetting (Bradbury 2006; Cumbana et al. 2007) is an effective method of cyanide removal.

In the lowland coastal Kenya where cassava is a dietary staple, boiling freshly harvested cassava roots is the norm. Cooke and Maduagwa (1999) reported a 55% reduction of bound cyanide by cooking of cassava. Similar figures (50-60%) were reported by Aalbersberg and Limalevu (1991) and 25-75% by Nambisan and Sundarsan (1985). The figures by Nambisan and Sundersan were dependent on cooking time and chips size, with smaller chips size recording highest cyanide losses.

Methods which use grating and crushing are very effective in removing cyanide because of the intimate contact in the finely-divided wet parenchyma between linamarin and the hydrolyzing enzyme linamarase, which promotes rapid breakdown of linamarin to hydrogen cyanide gas that escapes into the air (Cardoso et al., 2005). This in combination with wetting, fermentation and drying can reduce cyanide contents up to 99%.

Factors Affecting Cyanide Content of Cassava

1. **Cultivar**

Thousands of cassava cultivars exist and differ in their ability to tolerate pest and diseases, yield, nutritional and cooking qualities of food products. Cassava is propagated clonally from stem cuttings so there is minimal variation between individuals of one cultivar when grown under the same environmental conditions. All cassava cultivars contain cyanogenic glucosides however a wide variation in the concentration of cyanogens exists among different cultivars. This can range from 1 to 2,000 mg/kg (Cardoso et al., 2005, CIAT 2007). Cultivars with <100mg/kg hydrogen cyanide can be referred to as mild while those >100mg/kg can range from moderate to extreme (Wheatley et al., 1993).

2. **Climatic Conditions**

Cassava, a perennial shrub thrives in tropical and sub-tropical conditions. In general, the crop requires a warm humid climate. Temperature is important, as all growth stops at about 10ºC. The highest root production can be expected in the tropical lowlands, below 150 m altitude, where temperatures average 25-27°C, but some varieties grow at altitudes of up to 1500 m. The plant produces best when rainfall is fairly abundant, but it can be grown where annual rainfall is as low as 500 mm or where it is as high as 5,000 mm. The plant can stand prolonged periods of drought in which most other food crops would perish. This makes it valuable in regions where annual rainfall is low or where seasonal distribution is irregular. Cassava is drought resistant and grows well in poor soil (Java Cassava, 2007). The problem however is that cyanide content of cassava tends to increase during periods of droughts and /or prolonged dry weather due to water stress on the plant (Bokanga et al., 2004). Splittstoesser and Tunya (1992) reported that cassava grown in wet areas contain relatively lower amount of cyanide than those grown in drier areas.

3. **Fertilizer**

There is a general consensus that crop yields do increase with application of fertilizer. There is debate however on the relationship between addition of fertilizer and cyanide content of cassava.

Studies in the Philippines (Rolinda et.al, 2008) concluded that application of fertilizer does not significantly affect cyanide content. It further suggested that the amount of nutrient in the soil does not considerably contribute to the cyanogenic character of the cultivar. In Ethiopia, Endris (1977) suggested that the cyanogenic content of cassava roots were significantly reduced by potassium application.

4. **Harvesting**

Harvesting of cassava can be done throughout the year when the roots reach maturity. Maturity differs from one variety to another, but for food, the tubers can be harvested at 6 to 12 months (FAO, 1977) and can remain in the soil for up to three years after maturity (Lebot, 2009). Delayed uprooting causes sprouting during the rains resulting in a drastic fall in the starch content of the tubers. While the effect of harvesting method on cyanide is not clear, injuring the roots increases rate of post-harvest deterioration.

5. **Age of Cassava at Harvesting**

A study by Hidayat et al. (2002) on ninety nine variety of cassava showed that there is a significant correlation between cyanide potential of roots and leaves. The cyanide content was higher in younger leaves compared to older ones, suggesting that cyanide potential of roots drops as plant ages. This seems to agree with investigations by Chotineeranati, et al (2006). Cooke and Elba (1982) reported that the root parenchymal tissue and cortex were not significantly different between 6 and 14 months; they displayed peak concentrations at 6 and 14 months.

Cassava and Health

Despite the presence of these naturally occurring toxins, millions of people all over the world have been safely consuming cassava for hundreds of years. Usually, cassava is well processed before being consumed. Inadequate processing however may result in appreciable amounts of cyanogenic glycosides remaining and this may pose a public health risk (FSANZ 2008.). Konzo is a condition resulting from the excessive ingestion of cyanide compounds from inadequately prepared cassava and cassava products is characterized by irreversible paralysis of the legs in and other developmental disorders. They occurs mainly amongst children and women of child bearing age in Democratic Republic of Congo, where a reported 100,000 cases exist, Tanzania, Mozambique, Central African Republic, Cameroon and probably other countries (CCDN News, 2007). Tropical Ataxic Neuropathy (TAN) is another syndrome attributed to dietary cyanide exposure from inadequately prepared cassava. In contrast to Konzo, TAN is a progressive disorder that mainly affects older adults (CCDN News, 2008). It has been shown that different varieties of cassava have varying cyanide content and this quantity is affected by climatic conditions and other factors (Raji et al., 2007, CIAT, 2007). Studies in Africa have linked varying cyanide content in cassava to seasonal changes with higher concentrations of cyanide recorded in drought conditions. Iodine deficiency diseases are exacerbated by the intake of cyanogenic plants such as cassava.

The purpose of the study was to conduct a scientific assessment to determine ways along the food chain/commodity pathway to minimize the hydro cyanic acid content in cassava and its products and thus provide methods of meeting food quality standards requirement. This would be achieved by determining, through study and analysis, the effect on cyanide content based on:

- Agronomic factors (e.g. cultivars, stress,)
- Agroecological zone (CL 3, CL 4, and CL 5).
- Harvest/postharvest practices i.e. age at harvest.

Objective

To sustain and enhance the food security and livelihood of coastal lowland farmers and processors, by assisting them to successfully produce/ trade/ export their produce in compliance with food safety standards for cassava and cassava products.

MATERIALS AND METHODS

Sites: The study was conducted in Kilifi County in three agro ecological zones. They are coastal lowlands 3, 4 and 5 (CL3, CL4 and CL5). The locations were:

- CL3 Kikambala / Mtwapa
- CL4 Vitengeni / Madamani
- CL5 Bamba / Ganze.

Genotypes

The varieties studied and analysed were:

- Tajirika (improved)
- Shibe (improved)
- Karembo improved)
- Kibandameno (landrace)

Analysis of the varieties for CNP was carried out at:

- 5 months after planting
- 8 months after planting and
- 11 months after planting

Procedure

The sampling was done for 3 maturation stages; at 5, 8 and 11 months. Sampling was done at farmer's cassava plots where 3 samples of each varieties was collected for analysis. The samples of fresh roots were washed and labelled and be then transported to the SGS (Société Générale de Surveillance) LABS

Mombasa same day when the samples were still fresh. This was done thrice in a time span of 7 months. Data was analysed using the R software and SPSS was used for coding of the data.

RESULTS

The varieties under the trial, i.e. Kibandameno, Tajirika, Shibe and Karembo are generally low in cyanide levels. Kibandameno, the traditional variety is very palatable with desired qualities valued by the community. The variety had low levels of the CNP and high dry matter (DM) content. However, it is highly susceptible to diseases especially cassava mosaic and cassava brown streak diseases. Tajirika, Shibe and Karembo are the improved varieties whose levels of CNP is low, have high DM and which are resistant to those two diseases and are high yielders (50t/ha compared to KIB -5ton/ha).

Table 1: Mean and Standard Deviations of the cyanogenic potential levels (CNP)

AEZ	Mean (mg/kg)	STD
CL3	0.438	0.056
CL4	0.368	0.098
CL5	0.423	0.055
Variety		
Karembo	0.410	0.0565
Kibandameno	0.410	0.0928
Shibe	0.352	0.0906
Tajirika	0.400	0.077

In Table 1, in the agroecological zones there was significant difference in the cyanide levels while in the varieties there was no significant difference. The Karembo variety samples of the same age (11months) in clay loam soils in both CL 3 and 4 had cyanide levels of 0.45 and 0.37 mg/kg respectively. Kibandameno variety recorded the highest levels of CNP of 0.5 mg/kg in CL3 at 5 months and CL4 at 11 months in loamy soils and sandy loam respectively. Four samples of Shibe variety were collected in CL 4 and 5 giving the highest CNP level of 0.44mg/kg in sandy loam for 5 months old sample in CL5. The lowest levels were in loamy soils at 0.24 mg/kg at 5 months at CL4. Tajirika variety had the highest CNP level of 0.54mg/kg at CL4 in cassava sample harvested 11 months after planting while the lowest level of 0.28 was in CL4 in cassava sample harvested at 11 months in red loamy soils. Tajirika variety in CL3 at eleventh month after planting in clay soil has cyanide level of 0.39mg/kg. In CL3 at five months after planting in loamy soils for the Kibandameno variety, the cyanide level is recorded 0.5mg/kg while at 11 months after planting in the same soils and AEZ, the level is 0.33mg/kg. For Kibandameno, no sample was collected at 8 months after planting. In CL4, both Shibe and Tajirika varieties planted five months after planting in loamy soils recorded lower cyanide levels (0.24 and 0.33mg/kg respectively) compared with those planted eleven months ago in red loam soils (0.32 and 0.54 mg/kg).

At five months after planting, Tajirika, Shibe and Kibandameno varieties in CL5 recorded cyanide levels of 0.44 and 0.48mg/kg respectively in sandy loam soils. In the eleventh month, Shibe and Kibandameno varieties in the same soil type recorded 0.41 and 0.45mg/kg respectively. The Tajirika variety at eleventh month in sandy soils indicated cyanide levels of 0.32mg/kg in the CL5. In CL3, Kibandameno in loamy soils recorded 0.5 and 0.33 at five and eleven months respectively. An indication that the cyanide levels reduces as the plant ages. In the same AEZ, Tajirika, at 8 and 11 months in sandy soils recorded 0.45mg/kg showing no difference in the cyanide level. At eleven months, both Kibandameno and Tajirika in clay loam have a cyanide levels of 0.39mg/kg. In the CL4, Kibadameno, Tajirika and Shibe at eleven months and in red loamy soil had cyanide levels of 0.26, 0.28 and 0.32mg/kg respectively. At 5 months, Shibe and Tajirika cassava samples in CL4 had cyanide levels of 0.24 and 0.3 mg/kg respectively. In the CL5, Tajirika, Shibe and Kibandameno planted five months showed 0.44, 0.44 and 0.48mg/kg concentration of CNP while at 11 months the levels are 0.32, 0.41 and 0.45 respectively.

CONCLUSION

From the results**,** there is an indication that as the plant ages the levels of the CNP declines. This is supported by a study by Hidayat et al. (2002) on ninety nine variety of cassava that showed there is a significant correlation between cyanide potential of roots and leaves. The cyanide content was higher in younger leaves compared to older ones, suggesting that cyanide potential of roots drops as plant ages. This seems to agree with investigations by Chotineeranati et al (2006). Also noted is the continuous maintenance of the low cyanide levels by the four cassava genotypes for the entire period of the study. This suggests they are safe for consumption throughout their growth period.

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